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Respiratory Responses of the Blue Crab *Callinectes sapidus* to Long-Term Hypoxia

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Abstract. Blue crabs (*Callinectes sapidus*) were held in hypoxic (50–55 mm Hg) water for 7–25 days. Postbranchial blood PO_2 fell by about 80% within 24 h and then remained unchanged. Postbranchial blood total CO_2 increased within 24 h and remained elevated for the duration of the experiment. There was no change in postbranchial blood pH, osmolality, or Cl. Lactate, urate, and Ca^{+2} all raise the O_2 affinity of blue crab hemocyanin; by 25 days, blood lactate and urate had risen slightly, but Ca^{+2} had increased dramatically. Hemocyanin concentration had also increased by 25 days. At both 7 and 25 days there was an intrinsic increase in hemocyanin-O_2 affinity and a change in subunit composition. The highly adaptive homotropic change is believed to be due to an attendant shift in the proportions of two of the three variable monomeric hemocyanin subunits. Thus, both heterotropic and homotropic adaptations enhance blood oxygenation at the gill during long-term hypoxia.

Introduction

The respiratory response to long term hypoxia, defined here as exposure for three or more days, has been examined in six species of aquatic crustaceans: three crayfish (McMahon *et al.*, 1974; Dejours and Armand, 1980; Wilkes and McMahon, 1982a, b), a lobster (McMahon *et al.*, 1978), a crab (Burnett and Johansen, 1981), and a prawn (Hagerman and Uglow, 1985). In all cases, the initial response was hyperventilation, which resulted in a respiratory alkalosis. Subsequently, however, the response in different species became diverse. Blood pH either returned in full (Wilkes and McMahon, 1982a) or in large part (Butler *et al.*, 1978; McMahon *et al.*, 1978) to the normoxic level, or remained decidedly alkalotic for as long as 3–8 days (Dejours and Armand, 1980; Burnett and Johansen, 1981).

Crustacean hemocyanins (Hcs) typically have very large normal Bohr shifts; the quantity $\Delta \log P_{50}/\Delta pH$ is commonly near −1 (Mangum, 1980). Thus, the alkalosis, which had also been observed during acute hypoxia (Truchot, 1975; Burnett, 1979), would have the important respiratory consequence of raising blood O_2 affinity. The increases were observed, but were attributed by previous workers to the rise in blood pH. We now know that at least three other allosteric effectors, *viz.*, L-lactate (Truchot, 1980; Booth *et al.*, 1982), Ca^{+2} (Mangum, 1985) and urate (Morris *et al.*, 1985; Lallier *et al.*, 1987), also may increase HcO_2 affinity during acute hypoxia. The levels of these effectors in the blood during prolonged exposure, however, are not known.

Intrinsic changes in O_2 affinity of Hc in response to prolonged changes in environmental factors have recently been observed in both crayfish (Rutledge, 1981) and crabs (Mauro and Mangum, 1982; Mason *et al.*, 1983; Mangum and Rainer, 1988). In the blue crab, *Callinectes sapidus* Rathbun, salinity-induced changes are accompanied by shifts in the concentrations of two of the 5–6 subunits of the Hc polymers (Mason *et al.*, 1983). The changes in one of the two subunits fully explains the attendant shift in O_2 affinity (Mangum and Rainer, 1988).

Although an intrinsic molecular change would not be expected to occur during acute hypoxia, it might occur during long-term hypoxia. In the shrimp *Crangon crangon*, Hc levels increase sharply during prolonged hypoxia

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(Hagerman, 1986); a similar increase in the blue crab appears to hasten intrinsic molecular adaptation to a salinity change (Mason *et al.*, 1983). Therefore, we have examined the possibility that a change in net synthesis or degradation during hypoxia produces additional or replacement molecules that differ from those in normoxic animals.

The blue crab inhabits many bodies of water that are not invariably normoxic (Carpenter and Cargo, 1957; May, 1973; Garlo, 1979; Harper *et al.*, 1981; Turner and Allen, 1982). Lethal levels (PO_2 < about 50 mmHg) often kill animals that cannot escape from pots (Carpenter and Cargo, 1957). Free-ranging animals may even emerge into air (Loesch, 1960; Officer *et al.*, 1984), despite limited tolerance of it. In the Chesapeake Bay system, sublethal O_2 levels, still well below normoxia, are so widespread that crabs must encounter them for long periods. Water PO_2 in the range 50–100 mmHg is characteristic of the Chesapeake Bay for several months during the spring and fall (Officer *et al.*, 1984; Seliger *et al.*, 1985). In the summer, cyclical destratification in the channels produces sublethal hypoxia throughout the water column for several weeks at a time (Webb and D'Elia, 1980). Processes ranging from tidal flushing of the marshes, to seiching of the water in the channels, produce sublethal hypoxia in extensive areas of shallow water as well (Carpenter and Cargo, 1957; Axelrad *et al.*, 1976; Kemp and Boynton, 1980; Taft *et al.*, 1980; Malone *et al.*, 1986; MacKiernan, 1987).

We have determined the response of blood respiratory and osmotic variables of the blue crab *Callinectes sapidus* Rathbun to sublethal hypoxia. The treatments include acute exposure, more prolonged exposures similar to those employed in previous studies, and still more prolonged exposures designed to elicit intrinsic changes in the Hc molecule. We have measured all of the known physiological effectors of HcO_2 binding, both hetero- and homotropic.

Materials and Methods

Animals

Large (*ca.* 120–220 gm wet wt.), male, intermolt crabs were obtained from commercial watermen or collected by the first author near the Rhode River or the mouth of the Patuxent River in Maryland. They were returned to Fairfax, Virginia, and maintained in open containers (100–200 l) of natural, aerated water (500–530 mOsM, 21–23°C) for 4–7 days prior to the experimental hypoxia. Water osmolality was monitored frequently and distilled water added as needed; water pH was also monitored and kept above 7.9 by the addition of $NaHCO_3$. Crabs were fed thawed smelt twice a week throughout the control and experimental periods, but not within 24 h of sampling.

Design

The experimental protocol consisted of taking blood from the same crabs before, during, and following exposure to hypoxia. Insofar as possible, the design of paired observations on the same individuals was maintained, and the data were analyzed accordingly. The significance of changes in blood pH, PO_2, total CO_2, lactate, Cl, and osmolality was tested according to Student's *t*-test for ungrouped (paired) data as the mean of the differences of each value from the control for the same individual, the null hypothesis being that there was none. Because the same individuals were sampled repetitively, the blood samples were necessarily small (0.5 ml), thus insufficient volumes of many samples remained for the other measurements. The paired observations design could not be maintained for the analysis of Ca^{+2}, urate, and Hc concentrations; these values were analyzed by Student's *t*-test for grouped data (two samples). For measurements such as O_2 binding, which require a total of more than 0.5 ml of material, samples were pooled; the results were analyzed by regression. In these measurements, pooled samples for −1 and 7-day exposure were made up of blood from the same individuals, whereas that for 25-day exposure was composed of blood from different animals.

Hypoxia

Nitrogen gas was bubbled into the water to reduce the PO_2 from 140–155 to approximately 55 mm Hg in 3–4 h, and then the bubbling was stopped. Thereafter a slow, steady air flow was maintained, and N_2 was bubbled into the water only as needed to offset the air.

N_2 flow was regulated by a metering system that balanced N_2 against rising O_2. The system consisted of an O_2 electrode and meter (Instrumentation Laboratories Models 1703B and 113, respectively), the output of which provided the signal for a logic circuit that controlled an electric gas valve. The circuit was set to evaluate the output of the meter every 5 min and to open the valve if the PO_2 had increased above the set point of 50 mmHg. Thus, once the initial PO_2 of 50 mmHg was reached, further changes were confined to the ranges 50–55 mmHg and occurred slowly. The continuous airflow stirred the water and ensured that O_2 uptake by the crabs did not reduce water PO_2 below 50 mmHg.

Blood sampling

Postbranchial hemolymph samples for the determination of *in vivo* respiratory variables were withdrawn through holes in the carapace dorsolateral to the heart.

The holes had been drilled four or more days prior to the control period, and covered with latex rubber affixed with cyanoacrylate cement. On occasion, prebranchial hemolymph was also withdrawn from the base of one of the legs for the measurement of Cl, osmolality, and lactate.

Blood samples were withdrawn into iced syringes and immediately placed on ice to slow clotting. After determination of blood gas and acid-base variables, these samples were frozen for the remaining analyses. The samples for HcO_2 binding were kept cool and, with the exception noted below, unfrozen; O_2 binding measurements on blood from normoxic and hypoxic animals were made within a few days.

In vivo *variables*

Hemolymph pH was measured with a thermostatted glass capillary electrode (Radiometer G299A) and meter (Radiometer PHM 84). PO_2 was measured with a polarographic electrode (Radiometer E5046) and acid-base analyzer (PHM 72). Total CO_2 was determined in 50 μl samples with a Corning Model 965 CO_2 analyzer. Lactate was measured enzymatically (Sigma Procedure No. 826), with the modifications for Hc-containing blood developed by Graham *et al.* (1983). Osmolality was determined with a vapor pressure osmometer (Wescor Model 5100C).

Ca^{+2} activity was determined with a Radiometer electrode and PHM 84 meter, following 1:99 dilution with 0.05 Tris Maleate buffer, pH 7.6 (Mangum and Lykkeboe, 1979). Chloride was measured by electrometric titration (Corning Model 920).

We determined urate as the quinoneimine produced by digestion with uricase (Sigma Procedure No. 685), after first verifying that 100% of the urate added to test samples of blood could be recovered. Because Hc absorbs at 685 nm, the absorbance was measured in replicate, once with and once without the analytical reagents, and the interference of Hc was subtracted.

HcO_2 binding and Hc concentration

Hemolymph was declotted with a tissue grinder, centrifuged, and then dialyzed at 4°C for 24–28 h against a saline made up according to Mason *et al.* (1983). HcO_2 binding was determined by the cell respiration method, in which the deviation from a constant rate of O_2 depletion is used to estimate fractional oxygenation at the measured PO_2 (Mangum and Lykkeboe, 1979).

Before determining Hc concentration in the blood, we eliminated the effect of light scattering, dissociating the native polymers to monomeric subunits by dilution (1:39) with Tris HCl containing 50 m*M* EDTA. Absorbance of Hc was measured at 338 n*M* with a Milton Roy Spectronic 501 spectrophotometer; the concentration was calculated using the extinction coefficient for portunid Hc reported by Nickerson and Van Holde (1971).

Electrophoresis

Alkaline dissociation electrophoresis (Hames and Rickwood, 1981) of Hc monomers on polyacrylamide gel slabs was performed as described by Mangum and Rainer (1988). In the present case, aliquots of the three pools of blood used to compare O_2 binding in normoxic and hypoxic animals were run on the same gels, which were scanned with a Gelman Instruments Model 3372 integrating densitometer (modified for transparent media). Changes were estimated by comparing peaks of the variable subunits with that of an invariant subunit.

Results

The experiment was performed three times. The first hypoxic exposure period was 7 days, and samples were taken at −1 (control), 1, 4, and 7 days. The second and third exposure periods were 25 and 23 days, and samples were taken at −1, 7, 9, or 16, and 23 or 25 days. In the first experiment, the crabs were also sampled one day after the return to normoxic water.

Behavior and mortality

When ambient PO_2 fell to 50 mm Hg, most of the crabs became active and moved slowly around the aquarium, as reported by Lowery and Tate (1986); some crabs tried to climb out of the water. Elevated activity ceased within a few hours, and the animals became quiescent for the duration of the hypoxic exposure. They frequently buried in the sand lining the bottom of the aquarium.

Mortality was low. There was none during the first experiment and only 20% during the longer exposures. In our experience, this level would be low under normoxic conditions.

Hemolymph variables

Many of the data for normoxic animals are unexceptional (Table I, day −1), but pH and PO_2 are high relative to those in the literature for this species (*e.g.*, Weiland and Mangum, 1975; Mangum *et al.*, 1985). Our value for blood urate in normoxic animals is also considerably lower than that reported by Morris *et al.* (1986) for the crayfish *Austropotamobius pallipes* (0.35 m*M*), but it is similar to the figure (0.08 m*M*) found in the portunid crab *Carcinus maenas* (Lallier *et al.*, 1987). The low urate levels in the portunid bloods may explain the absence (Mangum, 1983) or small size (Truchot, 1975) of

Table I

Respiratory variables[1] in the hemolymph of blue crabs exposed to moderate hypoxia[2] for 7–25 days[3] (day −1 = control)

Duration[4] (days)	No. animals		PaO_2 (mm Hg)		pHa		$CaCO_2$ (mM)		Lactate (mM)		Ca^{+2} (mM)	Urate (mM)	[Hc] (g/100 ml)
	7	23–25	7	23–25	7	23–25	7	23–25	7	23–25	23–25	23–25	23–25
Day −1 (control)	8	9–11	98 ± 3	70 ± 8	7.81 ± 0.03	7.71 ± 0.03	2.3 ± 0.2	2.3 ± 0.4	0.01 ± 0.01	0.94 ± 0.09	6.73 ± 0.75	0.05 ± 0.02	3.11 ± 0.41
1	8		18 ± 4		7.79 ± 0.02		5.4 ± 0.3		0.08 ± 0.05				
4	8		15 ± 4		7.80 ± 0.04		5.1 ± 0.3		0.08 ± 0.06				
7	8	4–8	13 ± 2	22 ± 2	7.83 ± 0.03		4.6 ± 0.4	3.6 ± 0.2	0.09 ± 0.05		4.73 ± 0.75	0.03 ± 0.00	1.26 ± 0.11
Recovery	8		87 ± 10		7.77 ± 0.02		3.3 ± 0.2		0.00 ± 0.00				
9		6		19 ± 3		7.81 ± 0.06		2.6 ± 0.2		0.80 ± 0.09			
16		4		21 ± 3									
23–25		5–11		21 ± 4		7.76 ± 0.04		3.2 ± 0.3		1.79 ± 0.50	10.1 ± 0.5	0.14 ± 0.02	4.40 ± 0.19

[1] Top no. = mean, bottom no. = S.E.
[2] ~50–55 mm Hg, 21–23°C, 500–530 mOsM.
[3] Symbols: PaO_2 = postbranchial blood PO_2, pH = postbranchial blood pH, $CaCO_2$ = postbranchial blood total CO_2.
[4] Two columns under the first five headings represent different exposure periods, as indicated.

changes in HcO_2 affinity following dialysis of normoxic serum against a physiological saline.

Within 24 h of the onset of the 7-day hypoxic exposure in the first experiment, postbranchial blood PO_2 (PaO_2) fell by 80% and total CO_2 ($CaCO_2$) more than doubled while pH remained unchanged (Table I). The subsequent changes in these three variables are not significant ($P > .05$). The apparent increase in lactate is not significant ($P > .05$) if the difference at each sampling period is tested against zero. If the particular sampling period is disregarded and the maximum increase for each individual is tested against the null hypothesis, however, then the mean increase (0.18 ± .06 m*M*) is significantly greater than zero ($P < .01$). More important, this change is very small, indicating a highly aerobic condition. Within 24 h of return to normoxic water, control levels of postbranchial PO_2 were restored, although total CO_2 remained slightly elevated ($P < .02$).

In the second and third experiments, the same and longer periods of exposure (23–25 days) to hypoxia resulted in similar patterns of PO_2, pH, and total CO_2 (Table I). Once again blood lactate increased slightly, although in this case significantly ($P = .05$), regardless of how the data are grouped. At 25 but not 7 days, blood Ca^{+2} rose by a large amount ($P = 0.025$), blood urate rose significantly ($P < .001$), and Hc concentration increased by almost half ($P < .05$).

In none of the three experiments, did blood Cl or osmolality change, nor were there any coherent trends in these variables. The mean values (±S.E., n = 61) for all periods are 355 (±4) m*M* Cl and 756 (±5) mOsm.

HcO_2 binding and subunit composition

At the end of the 7-day period, a change in HcO_2 affinity had clearly occurred (Fig. 1, upper panel). The 95% confidence intervals around the regression lines describing the data for −1 and 7 days in Figure 1 do not overlap at any point (Table II). The slopes of regression lines (−0.97 ± 0.21 95% C.I. for normoxic and −1.11 ± 0.11 for 7 days, hypoxic animals), and thus the Bohr shifts, do not differ significantly.

The relationship between cooperativity (n) and pH of the decapod Hcs is usually quite complex, often reaching a maximum in the middle of the physiological pH range and showing lower values at the extremes. No very sensi-

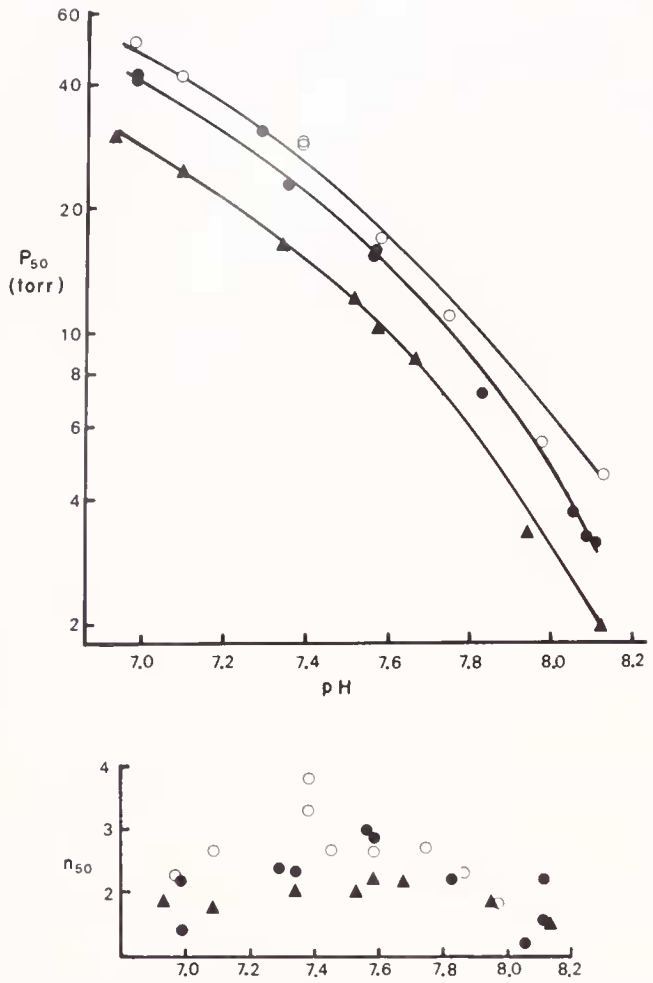

Figure 1. Oxygen binding by stripped Hc of normoxic (○), 7 days hypoxic (●), and 25 day hypoxic (▲) blue crabs. 25°C, 0.05 *M* Tris maleate buffered saline containing 494 m*M* NaCl, 16 m*M* KCl, 23 m*M* $CaCl_2$, 23 m*M* $MgCl_2$ 25 m*M* $(Na)_2SO_4$ and 2 m*M* $NaHCO_3$. Upper panel shows oxygen affinity (P_{50}), and lower panel shows the cooperativity at $PO_2 = P_{50}$ (n_{50}).

tive procedure for data analysis is available. In the lower panel of Figure 1, cooperativity seems to decrease at 7 days, but the mean values are not significantly different ($P = .10$). A Mann-Whitney U test also did not distinguish a significant change.

There was a clear change in the subunit composition of the Hcs (Fig. 2). Specifically, subunits 3, 5, and 6 decreased in concentration relative to subunit 4, which has remained invariant in samples taken, by now, from more than 500 individuals (Mangum, unpubl. obs.; Rainer, 1988).

After 25 days of hypoxia, HcO_2 affinity increased further (Fig. 1). The 95% confidence interval around a regression line describing O_2 affinity does not overlap those for control or 7-day hypoxic animals in any part of the pH range (Table II). The slope of the regression line describing the 25 day data (−1.00 ± 0.17 95% C.I.) does not differ from the other two. In this case, the mean value for cooperativity (1.95 ± 0.06 S.E.) of the Hc from 25 day hypoxic animals (Fig. 1) differs significantly ($P = .02$) from that for control and 7 day hypoxic animals (2.41 ± 0.14).

All three variable subunits decreased further in concentration relative to subunit 4 (Fig. 2). Indeed the presence of subunit 3, which is sometimes completely absent (Mason *et al.*, 1983), is dubious. By the end of 25 days of hypoxia, the concentration of subunit 6 had dropped from the highest in the control period to rank fourth; no. 5 had dropped from second to third; and no. 3 had dropped from clearly present to undetectable, or nearly so. The two weak bands appearing between peaks 1 and 2 of the Hc from hypoxic crabs (Fig. 2B, C) are usually not present: they are not copper containing and have no influence on oxygen binding (Mangum and Rainer, 1988).

Although the effects of elevated Ca^{+2} (and L-lactate) on *C. sapidus* Hc are well known (*e.g.*, Booth *et al.*, 1982; Mangum, 1983; Mason *et al.*, 1983; Johnson *et al.*, 1984), those of urate are not. Therefore we used the small amount of (frozen) blood remaining after the measurement of extrinsic co-factors to examine urate sensitivity. Figure 3 shows that small quantities of urate clearly raise O_2 affinity of the Hc of animals exposed to hypoxia for 25 days, with its altered subunit composition. The positions (but not slopes) of regression lines describing the data for 0, 0.55, and 2.35 m*M* urate all differ at $P = 0.05$. Although the data suggest little further difference beyond 1.17 m*M*, the small number of observations permitted by the volume of material available mandates some caution on this point.

We emphasize that, unlike the measurements in Figure 1, those in Figure 3 were made on Hcs that had been frozen for several months. As mentioned earlier (Mangum, 1983), freezing does not usually influence P_{50} (see also Morris, 1988), at least if the Hc retains its native

Table II

Ninety-five percent confidence intervals around semilogarithmic (log Y) regression lines fit to P_{50} data in Figure 1

pH	Control ($r^2 = 0.946$)	7-day hypoxia ($r^2 = 0.994$)	25-day hypoxia ($r^2 = 0.965$)
7.0	57.2–60.5	49.7–53.5	31.0–33.7
7.2	36.6–38.6	30.5–32.7	19.6–21.2
7.4	23.4–24.6	18.7–20.0	12.4–13.4
7.6	15.0–15.7	11.6–12.2	7.86–8.46
7.8	9.54–10.1	7.01–7.48	4.96–5.35
8.0	6.09–6.44	4.29–4.59	3.13–3.39
8.2	3.88–4.12	2.62–2.81	1.97–2.15

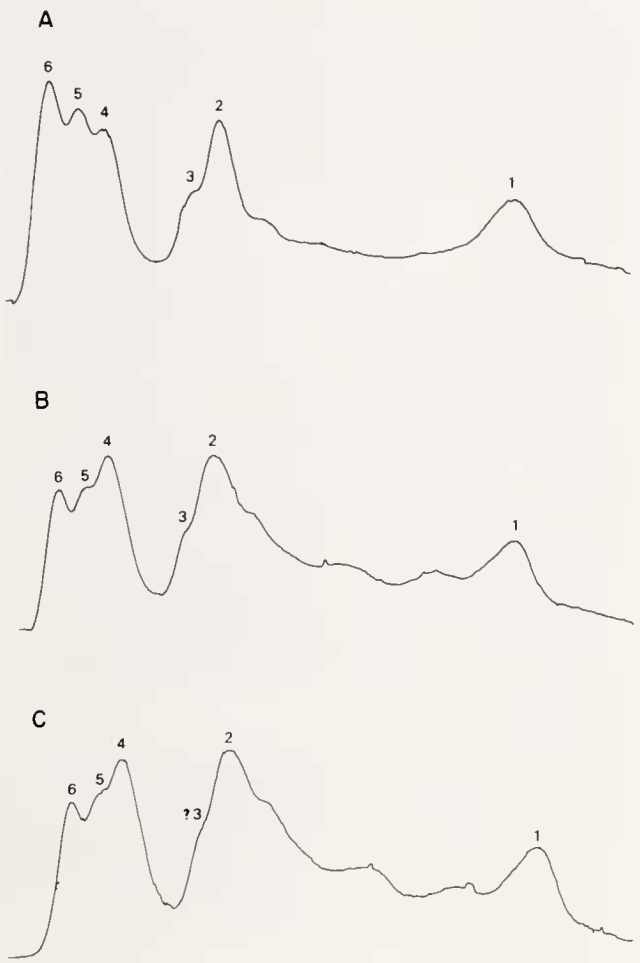

Figure 2. Densitometer scan of slab gels, showing native subunits (numbered peaks) of blue crab Hc separated by charge (subunit 1 is at the anodal end). The Hc applied to the gels was from the same samples from which data were collected for Figure 1. A. Normoxic. B. 7-day hypoxic. C. 25-day hypoxic. Subunit 3 in C is dubious.

optical properties. The control values in the two figures are essentially identical (95% confidence intervals around regression lines broadly overlap). Because freezing frequently influences cooperativity, however (Mangum, 1983; S. Morris, pers. comm.), we did not analyze the cooperativity of the thawed samples. Morris *et al.* (1986) found no effect of urate on cooperativity.

Discussion

Blood pH, PO_2, and CO_2

In view of the unanimity of previous reports of blood alkalosis accompanying hypoxia of virtually any duration in crustaceans, we were surprised to find none in the present experiments. Hyperventilation and alkalosis are not always precisely correlated; in the crayfish *O. rusticus*, ventilation returns to control levels while blood pH is still elevated (Wilkes and McMahon, 1982a). But all reports agree that blood pH rises at some point. In fact, in severely hypoxic *C. maenas*, Lallier *et al.* (1987) reported a pH increase of more than 0.3 units accompanying an increase in lactate of 25 m*M*, despite no change in base. In other investigations of *C. sapidus*, we have either found (Pease *et al.*, 1986), or not found (Mangum and Weiland, 1975, and unpubl. obs.), a hypoxic alkalosis. The response in this species is apparently highly labile, for reasons that are presently unclear. The increases in lactate observed here seem too small to offset a respiratory alkalosis brought about by vigorous hyperventilation (Pease and deFur, 1987). Further increases in pH and PO_2 might have been precluded because ventilation was already high. Elevated ventilation during the control period could have arisen from sensory stimulation (McDonald *et al.*, 1977) and been unrelated to ambient PO_2.

Extrinsic modulation of HcO_2 affinity

In many crustaceans L-lactate is a physiologically important modulator of HcO_2 affinity, both during exercise and hypoxia (Booth *et al.*, 1982; Graham *et al.*, 1983) and very severe environmental hypoxia (Lowery and Tate, 1986; Lallier *et al.*, 1987). The small increases observed here would raise HcO_2 affinity at physiological pH by less than 1 mmHg. The increase in urate would raise HcO_2 affinity by a similarly small amount. In contrast, Ca^{+2} may be an important effector after 23–25 (but not 7) days, by which time the increase in Ca^{+2} would raise HcO_2 affinity by more than 5 mmHg.

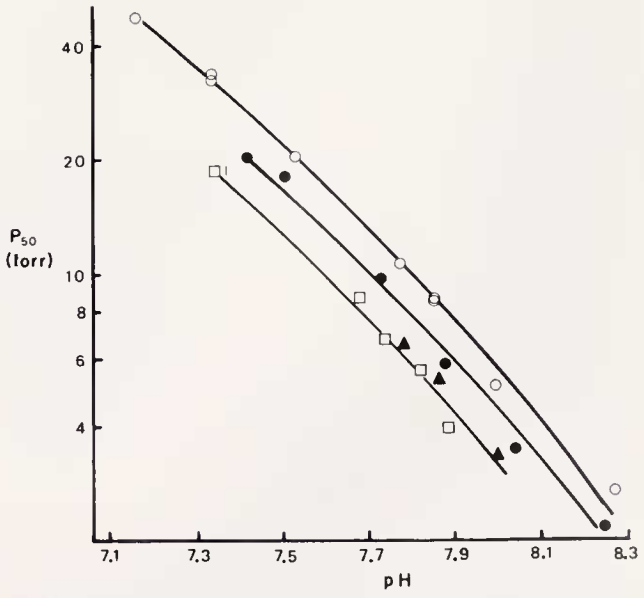

Figure 3. Effect of urate on O_2 affinity of stripped Hc from hypoxic animals. Conditions as in Figure 1. (○) no urate; (●) 0.57 m*M*; (▲) 1.15 mM; and (□) 2.35 m*M*.

These conclusions, inferred from the data in Figure 3 and those of Mason *et al.* (1983) and Johnson *et al.* (1984), assume that the two organic effectors act completely independently and cumulatively, which is not entirely true (S. Morris, pers. comm.). The interaction of Ca^{+2} with the organic effectors is included in the present data for HcO_2 affinity because the actions of urate and lactate were determined in the presence of Ca^{+2}. The interaction of urate and lactate would further diminish their effects, albeit by a small amount.

Intrinsic adaptation of HcO_2 affinity

Estuarine (and also normoxic) blue crabs transferred to high salinity in the laboratory show a rapid decrease in Hc concentrations. Concomitantly, HcO_2 affinity increases and the levels of subunits 3 and 5 decrease, closely resembling the Hc in animals freshly caught at a seaside location. Subunit 6 remains unchanged. In seaside (and normoxic) animals transferred to low salinity, the Hc concentration rapidly increases while the HcO_2 affinity decreases (Mason *et al.*, 1983). The intrinsic change opposes the effects of salinity-induced changes in extrinsic co-factors.

In initial samples freshly taken at the estuarine and seaside localities, the vast majority of animals exhibited the molecular phenotype associated with the comparable acclimation salinity in the laboratory (C. P. Mangum and G. Godette, unpubl. obs.; Rainer *et al.*, 1985). However, subunit 6 was variable in both samples, implicating another environmental or physiological effector unrelated to salinity *per se*. Moreover, when the field study was enlarged, no clinal variation of the three subunits was obvious along a salinity gradient between the two localities (Rainer, 1988); these findings also suggest a confounding variable.

Under the same ionic conditions, the O_2 affinities of blue crab Hcs composed of different combinations of the variable chains indicate that the levels of subunits 3 and 6 both influence oxygen binding; the effects of variation in subunit 5 are not entirely clear (Mangum and Rainer, 1988). Changes in subunit 3 (alone) can fully explain the difference between seaside and estuarine animals. However, changes in subunit 6 (alone), smaller than those observed here, significantly alter HcO_2 affinity by almost 20% at physiological pH. Although there is no difference between an Hc with low levels of only subunit 3, and one with low levels of both 3 and 5, an Hc with low levels of 5 alone has not been examined.

The present results suggest that blue crab Hc is intrinsically adaptable to prolonged hypoxia as well as to salinity. The adaptation may be expedited by an increase in Hc concentration, which is clear at 25 days, and it is accompanied by changes in the same three subunits already known to be variable. A decrease in concentration of subunits 3 and 6 during hypoxia has the same effect as that of decreasing either alone or in combination; *i.e.*, increasing O_2 affinity (Mangum and Rainer, 1988). The present findings suggest that, while subunits 3 and 5 respond to a change in both salinity and oxygen, subunit 6 responds only to oxygen. The changes in subunits 3 and 5 as a result of hypoxia were much smaller than the salinity-induced changes, but the changes in P_{50} were about the same in the two groups, at physiological pH. The smaller changes in the amounts of subunits 3 and 5 in hypoxia may be due to lower levels at the onset of hypoxia (for comparison see fig. 1 in Mangum and Rainer, 1988). The oxygen-induced change in subunit 6, however, was much larger than observed by Mangum and Rainer (1988). A greater change in subunit 6 may offset a smaller change in subunit 3, and the intrinsic adaptation of HcO_2 affinity to hypoxia may involve the change in subunit composition. Moreover, we suggest that the variation of subunit 6 in nature is related to hypoxia, which does not vary along a salinity gradient in a simple fashion.

Finally, the increase observed here in Hc concentration occurs widely in hypoxic crustaceans. In different species its magnitude may be much greater, it may occur in a far shorter period, and it may occur at a much lower temperature (Hagerman and Oksama, 1985; Hagerman and Uglow, 1985; Hagerman, 1986). It will be interesting to learn whether intrinsic molecular adaptability is similarly widespread.

Acknowledgments

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